Chapter 5
Regulatory Protein-Protein Interactions in Primary Metabolism: The Case of the Cysteine Synthase Complex

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Abstract Sulfur is an essential nutrient for plant growth and development. In plant sulfur assimilation, cysteine biosynthesis plays a central role in fixing inorganic sulfur from the environment into the metabolic precursor for cellular thiol-containing compounds. A key regulatory feature of this process is the physical association of the two enzymes involved in cysteine biosynthesis (serine acetyltransferase, SAT, and O-acetylserine sulfhydrylase, OASS) to form the cysteine synthase complex. Physiologically, this multienzyme complex acts as a molecular sensor in a regulatory circuit that coordinates sulfur assimilation and modulates cysteine production. Here we focus on aspects of the protein-protein interactions in the plant cysteine synthase complex and how formation of the complex has been studied. In addition, we summarize the initial efforts to understand the structural, kinetic, and thermodynamic basis for association of SAT and OASS in the multienzyme assembly.

1 Introduction

In plants and bacteria, cysteine biosynthesis is the final pathway in the conversion of inorganic sulfur into a chemically stable organic compound. Two enzymes catalyze the reactions of this pathway (Rabeh and Cook 2004, Wirtz and Droux 2005, Kopriva 2006). In the first step, serine acetyltransferase (SAT, EC 2.3.1.30) generates O-acetylserine by transferring acetate from acetyl-coenzyme A to serine to form O-acetylserine. During the second step, O-acetylserine sulfhydrylase (OASS, EC 4.2.99.8) uses pyridoxal phosphate (PLP) as a cofactor to yield cysteine from O-acetylserine and sulfide. Cysteine biosynthesis is the metabolic link between sulfur assimilation and the myriad of sulfur-containing molecules in the cell. For example, this pathway provides essential metabolites for production of glutathione, a key regulatory agent of intracellular redox environment during abiotic and biotic stresses.

Cysteine biosynthesis in plants also contributes to regulating sulfur assimilation by influencing the expression of genes involved in sulfur uptake and assimilation

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and by modulating enzymatic activities in response to sulfur demand (Hell and Hillebrand 2001, Saito 2004, Kopriva 2006, Wirtz and Hell, 2007). This mechanism involves the physical association of SAT and OASS to form the hetero-oligomeric cysteine synthase complex; however, little is known about the molecular mechanism of how these proteins associate and the three-dimensional architecture of the complex. This chapter focuses on aspects of protein-protein interactions in the plant cysteine synthase complex and how its formation has been studied.

2 Overview of the Cysteine Synthase Complex

Kredich and co-workers (1969) first described the cysteine synthase complex after isolating it from *Salmonella typhimurium*. Initially, metabolic channeling was suggested as the function of this macromolecular assembly, but later studies demonstrated this was not the case (Cook and Wedding 1977). Nearly two decades after the bacterial enzyme complex was identified, Nakamura and Tamura (1990) identified the complex in plants. Subsequently, formation of the cysteine synthase complex by interaction of SAT and OASS from plants and bacteria has been shown using a variety of experimental approaches, including size-exclusion chromatography, yeast two-hybrid analysis, surface plasmon resonance, fluorescence spectroscopy, and calorimetry. Efforts to map the protein-protein interaction regions in the complex indicate that the C-terminus of SAT plays a critical role in association with OASS (Bogdanova and Hell 1997, Mino et al. 1999, 2000, Wirtz et al. 2001, Francois et al. 2006, Kumaran and Jez 2007). Recently, determination of the x-ray crystal structures of the *Haemophilus influenzae* OASS (HiOASS) and *Arabidopsis thaliana* OASS (AtOASS) in complex with peptides corresponding to the C-termini of the SAT from each organism and protein-protein interaction studies revealed that the OASS active site forms the SAT interaction site (Bonner et al. 2005, Huang et al. 2005, Campanini et al. 2005, Zhao et al. 2006, Francois et al. 2006, Liszewska et al. 2007). Although individual components of the complex have been identified and the biochemical properties of SAT and OASS well studied, the mechanism of assembly and structure of the cysteine synthase complex remains to be defined.

2.1 Protein-Protein Interaction Modulates the Activities of SAT and OASS

The cysteine synthase complex is a macromolecular assembly that plays a regulatory role in plant sulfur assimilation with protein-protein interactions between SAT and OASS modulating each enzyme's activity. Functional studies have provided a wealth of information on the kinetic and chemical mechanisms of both SAT and OASS. The biochemical properties of the plant and bacterial SAT have been extensively examined (Noji et al. 1998, Leu and Cook 1994, Droux et al. 1998, Johnson

et al. 2004a, 2004b). Likewise, the reaction mechanism of OASS has been dissected in detail (Rabeh and Cook 2004).

Interestingly, the activity of each protein changes upon association in the plant cysteine synthase complex. SAT activity is up to 20-fold higher in the complex than as an isolated protein (Droux et al. 1998, Berkowitz et al. 2002, Hell et al. 2002, Wirtz and Droux 2005). In contrast, OASS activity is almost completely abrogated when complexed with SAT (Kredich et al. 1969, Droux et al. 1998, Berkowitz et al. 2002). Furthermore, in plants, unbound SAT rapidly loses its activity in the absence of OASS with maximal SAT activity for cysteine synthesis requiring a nearly 400-fold excess of OASS activity (Droux et al. 1998). Overall, these findings led to the conclusion that association of SAT and OASS into the cysteine synthase complex is a prerequisite for optimal flux of cysteine synthesis in vivo (Hell and Hillebrand 2001).

2.2 *Role of the Complex in a Regulatory Circuit Controlling Sulfur and Cysteine Metabolism*

SAT and OASS associate to form the cysteine synthase complex under sulfur-sufficient conditions in plants (Droux et al. 1998, Saito 2004) (Fig. 5.1). As part of the complex, the bound form of OASS retains very little activity, but SAT activity is enhanced. The mechanism for increased SAT activity remains to be established, but structural studies have revealed how OASS activity is reduced (see Section 5.3.1.). Complex formation results in the production of the pathway intermediate O-acetylserine (OAS). Under sulfur-sufficient conditions, free OASS catalyzes cysteine formation from OAS and sulfide. If intracellular sulfur levels are low, then OAS accumulates, as free OASS is unable to produce cysteine due to a lack of sulfide. Elevated levels of OAS promotes dissociation of the cysteine synthase complex and effects sulfur metabolism, as the higher OAS concentration activates expression of genes encoding sulfate transporters, ATP sulfurylase, OASS, and SAT (Smith et al. 1997, Koprivova et al. 2000, Hopkins et al.

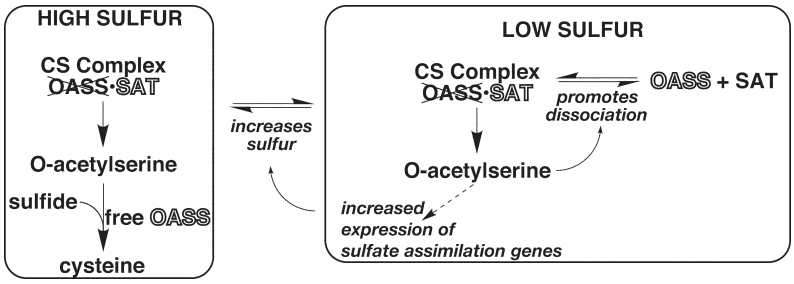

Fig. 5.1 Model for regulation of sulfur assimilation and cysteine synthesis by the plant cysteine synthase complex. Highly active forms of OASS and SAT are indicated by the outlined text. The inactivated form of OASS in the complex is indicated with an "X." Modified from Hell and Hillebrand 2001.

2005). This leads to increased sulfur uptake and assimilatory reduction. As sulfur levels elevate, free OASS begins to catalyze cysteine formation, which reduces OAS levels. Decreased concentration of OAS allows for association of SAT and OASS, which results in activation of SAT and resumption of cysteine biosynthesis (Hell and Hillebrand 2001).

2.3 Initial Structural Investigations of the Cysteine Synthase Complex

Although the three-dimensional structure of the cysteine synthase complex remains to be solved, x-ray crystal structures of the OASS from bacteria (Burkhard et al. 1998, Claus et al. 2005) and *Arabidopsis thaliana* (Bonner et al. 2005) and the bacterial SAT (Olsen et al. 2004, Pye et al. 2004) are available. The OASS from plants and bacteria form stable homodimers with a molecular weight of 68-73 kDa (Burkhard et al. 1998, Bonner et al. 2005, Claus et al. 2005) (Fig. 5.2a). Each OASS monomer consists of two α/β structural domains. The smaller N-terminal domain (residues 45-150 in AtOASS) contains a central four-stranded parallel β-sheet flanked by four α-helices. The larger C-terminal domain (residues 3-44 and 151-303 in AtOASS) centers on a six-stranded β-sheet surrounded by four α-helices. The active site is located at the interface of these domains and is defined by the location of the PLP cofactor. The three-dimensional structures of bacterial SAT have been solved using x-ray crystallography (Olsen et al. 2004, Pye et al. 2004). SAT functions as a hexameric protein with a molecular weight of 180-200 kDa (monomer Mr 30-35 kDa). The overall structure consists of a largely α-helical N-terminal domain and a C-terminal domain dominated by a left-handed β-helix structure consisting of 14 β-strands forming five coils of the helix (Fig. 5.2b). The N-terminal domain is involved in interaction with other monomers in the structure with the C-terminal domain containing the active site of the enzyme.

Relatively little information is available about the structural and molecular properties of the cysteine synthase complex from either plants or bacteria. Kredich et al. (1969) determined a total molecular weight of 310 kDa for the *S. typhimurium* hetero-oligomeric complex. With estimated weights of 160 kDa for the SAT homo-oligomer and 68 kDa for the OASS homodimer, it was assumed that two OASS dimers bind to one SAT hexamer; however, so far no precise analytical data are available to support this assumption. Biochemical and molecular biology approaches established that the 10 to 20 amino acid C-terminal tail of SAT is essential for association with OASS (Bogdanova and Hell 1997, Mino et al. 1999, 2000, Wirtz et al. 2001, Zhao et al. 2006, Francois et al. 2006, Kumaran and Jez 2007). Moreover, protein crystal structures of AtOASS and HiOASS in complex with peptides corresponding to the C-termini of their cognate SAT reveal that the OASS active site forms the binding site for the C-terminus of SAT (Huang et al. 2005, Francois et al. 2006). Nonetheless, how the two full-length proteins interact to form the complex remains to be elucidated.

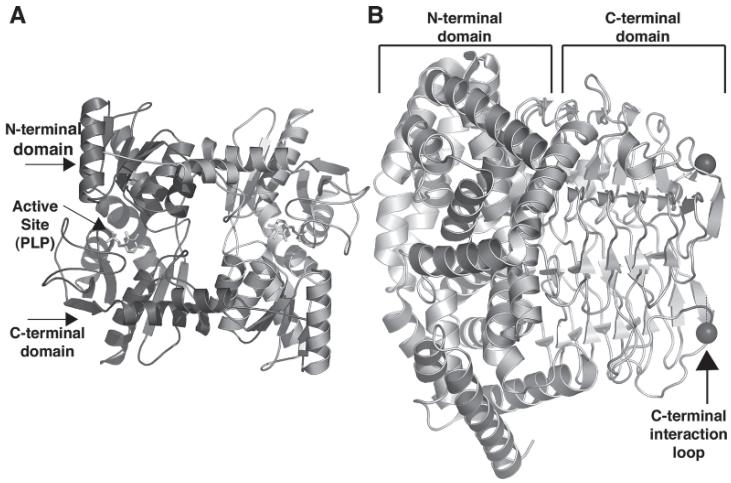

Fig. 5.2 (A) Ribbon diagram of the AtOASS dimer (Bonner et al. 2005). Each monomer is shaded in different grays. The location of the PLP cofactor (drawn as a stick model) and active site are indicated. (B) Ribbon diagram of one trimer of the *E. coli* SAT (Pye et al. 2004) with the N- and C-terminal domains indicated. The C-terminal loop believed to interact with OASS is indicated by the three spheres.

3 Methods for Studying Protein-Protein Interactions in the Cysteine Synthase Complex

Structural, kinetic, and thermodynamic information is required to completely understand how SAT and OASS assemble to form the cysteine synthase complex. While trying to characterize protein-protein interactions, experimentalists encounter two major difficulties. First, biophysical methods require the purification of sufficient amounts of stable protein to perform the necessary studies. The second challenge is to find optimal solution conditions under which the interacting proteins are stable, maintain their native structure, and do not undergo any non-native oligomerization. All of the possible methods and approaches to solve these problems will not be discussed here; however, assuming one has overcome such obstacles, complete understanding of any macromolecular interaction requires a multifaceted approach to obtain quantitative information about the system. In this section, we discuss quantitative approaches for dissecting protein-protein interactions in the cysteine synthase complex.

3.1 Crystallographic Analysis of the Interaction between AtOASS and the AtSAT C-Terminus

The ultimate goal of studying protein-protein interactions is to understand the recognition principles involved in macromolecular assembly. An essential part of this process

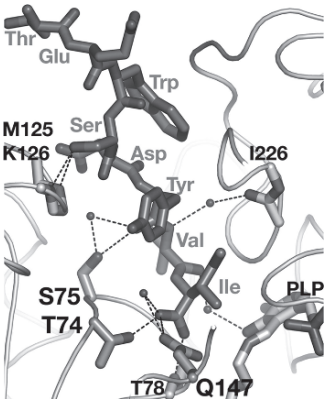

Fig. 5.3 Interactions in the AtOASS C10 peptide binding site. All modeled residues of the C10 peptide are shown and labeled. Side chains of residues interacting with the C10 peptide are indicated. Water molecules are shown as spheres. Dotted lines represent hydrogen bonds.

involved structure determination by protein crystallography, which provides a detailed view of spatial arrangement of atoms and interactions. The 2.5 Å resolution x-ray crystal structure of AtOASS in complex with a peptide corresponding to the 10 C-terminal residues of AtSAT (C10 peptide) revealed the molecular mechanism for downregulation of OASS activity in the plant cysteine synthase complex (Francois et al. 2006). The overall structure of the AtOASS C10 peptide complex is similar to that of AtOASS and clearly shows 8 of 10 amino acids in the C10 peptide bound to AtOASS, with the N-terminal two residues disordered. The C10 peptide binds at the AtOASS active site in an extended conformation with the C-terminal isoleucine of the peptide positioned near the PLP and the remaining residues occupying a cleft that forms the active site entrance (Fig. 5.3). Thus, changes in OASS activity upon interaction with SAT results from blocked access to the catalytic center of the enzyme. Although structural studies indicate that several key residues from both AtOASS and the C10 peptide contribute to binding, the relative contribution of these individual residues to complex formation and activity cannot be accessed without functional analyses. Thus, to test the specific role of these residues and to quantify their relative contribution, solution-based binding and activity studies complement crystallographic data.

3.2 Mapping Hot-Spots That Play Dual Roles in Cysteine Biosynthesis Using Mutagenesis and Calorimetry

Previously, crystallographic studies provided a guide for extensive site-directed mutagenesis, steady-state kinetic characterization, and ligand binding analysis of AtOASS (Bonner et al. 2005). A similar approach was adopted to dissect the role of residues in binding the C10 peptide using isothermal titration calorimetry (ITC) (Francois et al. 2006). In AtOASS, Thr74, Ser75, and Gln147 form an extensive interaction network with the C-terminal isoleucine of the C10 peptide, locking it in the active site/peptide-binding pocket. Mutation of each of these three residues to alanine decreased the OASS activity more than 1,000-fold (Bonner et al. 2005).

Similarly, site-directed mutations of these residues also effected C10 peptide binding, as determined by ITC. Compared to wild-type enzyme, the T74S, S75A, S75T, and Q147A mutants all displayed lower affinity for the C10 peptide with up to 100-fold increases in K_d values (Francois et al. 2006). As these residues form hydrogen bonds with the C10 peptide through their side chains, loss of these interactions results in decreased binding affinity. Interestingly, mutation of these residues also had a profound effect on the stoichiometry of complex formation. Each mutant enzyme bound only one C10 peptide in contrast to the 2:1 stoichiometry (i.e., two moles of C10 peptide bound to one mole of AtOASS dimer) observed for wild-type AtOASS. These mutational and binding studies suggest that protein-protein interaction in the cysteine synthase complex and the catalytic activity of OASS rely on a common set of critical or "hot-spot" residues. Additional studies are needed to completely map the hot-spot residues beyond the OASS active site that dictate interaction with SAT in the plant cysteine synthase complex. (More information about ITC and the analysis of the thermodynamics of binding interactions is given in section 5.4.)

3.3 Fluorescence-Based Assays to Monitor Complex Formation and Allostery

An alternative method to ITC for probing binding events is to employ fluorescence-based assays that depend on either intrinsic protein fluorescence or that of a chromophore. In addition to determining binding constants, equilibrium binding studies performed under very high affinity solution conditions can provide the absolute stoichiometry of an interaction.

The active site of OASS contains a natural fluorphore – the PLP cofactor (Campanini et al. 2005, Kumaran and Jez 2007). Excitation of PLP at 412 nm generates a fluorescence emission spectrum with a maximum around 505 nm. Binding of either SAT or C10 peptide at the OASS active site can be determined by monitoring changes in the intrinsic fluorescence emission signal of PLP. Upon addition of either SAT or C10 peptide, the relative fluorescence of PLP in the OASS active site increases. Titrations of AtOASS with the C10 peptide show a maximal 1.8-fold change in emission signal at saturation, indicating that a maximum binding of two C10 peptides bind per AtOASS dimer (Kumaran and Jez 2007). Further, the C10 peptide binds to AtOASS with very high affinity, i.e., K_d = 10 nM for each binding site.

Structural studies reveal that AtOASS is a symmetric dimer with two identical active sites, but analysis of C10 peptide binding to AtOASS using a fluorescence-based assay revealed that high concentrations of NaCl affects binding of the peptide (Kumaran and Jez 2007). Binding of the C10 peptide to AtOASS is only weakly sensitive to NaCl concentration below 0.3 M, suggesting that the binding energy is derived from mainly nonelectrostatic interactions. In contrast, at higher salt concentrations the two binding sites in the dimer exhibit completely different binding affinities. The binding of the first C10 peptide at the active site of one monomer decreases affinity for binding of the second peptide at the other active site through

negative cooperativity between each subunit. Allosteric structural changes and inhibition of the bacterial OASS occur at less than 0.05 M NaCl (Tai et al. 2001). It is conceivable that chloride may be a potential allosteric modulator of the plant OASS; however, the biological function of this allosteric mechanism in plants is unclear.

3.4 Surface Plasmon Resonance (BIAcore) Analysis of the OASS-SAT Interaction

Unlike calorimetry and fluorescence assays that measure equilibrium binding constants, surface plasmon resonance or biomolecular interactions analysis (BIAcore) is used to determine the kinetic association and dissociation rates of a binding interaction. A BIAcore instrument monitors molecular interactions by surface plasmon resonance, which detects changes in the mass of molecules at an interface by probing changes in refractive index (Malmquist 1993). For these experiments, one interaction partner is either chemically immobilized or noncovalently tethered to a gold-film sensor chip. While the second interaction partner flows over the sensor chip and complexes form and dissociate at the metal surface, the refractive index of the interface changes. The time-dependent change in the refractive index is used to determine association and dissociation rate constants.

Berkowitz and co-workers (2002) used this method to evaluate formation of the cysteine synthase complex with the mitochondrial isoforms of AtSAT and AtOASS and dissociation of the complex by O-acetylserine. Using a 1:1 binding model, they determined a K_d = 25 nM for the complex formation. In addition, they analyzed the effect of OAS on dissociation of the complex, which showed a half-maximal rate at 77 µM with strong positive cooperativity. Importantly, this value is in the range of OAS concentrations found in the cell and is consistent with the regulatory model in which changes in OAS levels affect formation of the complex and modulates cysteine synthesis.

4 Thermodynamic Basis for Formation of the Plant Cysteine Synthase Complex

Although structural and binding experiments revealed the specificity of interaction and quantification of binding affinity in the plant cysteine synthase complex, these studies alone cannot provide information about the thermodynamic forces that define the binding affinity and drive interaction of SAT and OASS. Understanding the thermodynamic basis for formation of the cysteine synthase complex would provide insight into the different physical forces that optimize complex formation. Complete thermodynamic characterization of a binding interaction requires (1) that the affinity and stoichiometry of the interaction are known, (2) determination of the net enthalpy change upon interaction (i.e., the heat change that occurs upon breaking or forming bonds), and (3) the entropy change that results from changes in order

of the system. Such an analysis depends on using calorimetry to directly measure the heat changes during a molecular recognition process and allows for the determination of all three thermodynamic parameters (ΔH, ΔS, and ΔG) in one experiment (Stites 1997). Since many texts describe the principles, design, and application of ITC, we will not discuss those details here.

4.1 AtOASS is a Symmetric Dimer: Structurally and Thermodynamically

Crystallographic and fluorescence binding experiments demonstrate that each subunit of AtOASS tightly binds a C10 peptide molecule; however, the energetics governing complex formation can only be probed using ITC. In an ITC experiment, the instrument detects the changes in heat occurring upon injection of small volumes of one ligand (C10 peptide) into a chamber containing the interaction partner (AtOASS) (Fig. 5.4a). Analysis of this data yields a binding isotherm (Fig. 5.4b) and estimates of the thermodynamic properties of the interaction.

ITC binding studies demonstrates that the two C10 peptide binding sites of the AtOASS dimer are functionally independent and bind the peptide with very similar affinity: $K_{eq} = 6.8 \times 10^7$ M^{-1} ($K_d = 14.7$ nM) (Kumaran and Jez 2007). The enthalpy change associated with binding at a single active site is ΔH = -12.7 kcal mol^{-1}. Although binding of OAS to the plant OASS can exhibit positive cooperativity (Droux et al. 1998), which suggests that structural changes during the catalytic cycle may mediate allosteric communication between the active sites in the homodimer, analysis of the AtOASS•C10 peptide interaction shows no evidence for any cooperativity between subunits at 25 °C and low (< 0.3 M) NaCl concentrations. Thus, the two binding sites of AtOASS are symmetric in nature, so that C10 peptide binding at one site is independent of binding at the second site over broad temperature (10-30 °C) and salt ranges (0.02-0.3 M NaCl). These results agree with crystallographic studies of AtOASS alone and in complex with the C10 peptide that shows no global structural changes occur upon peptide binding.

4.2 Effect of Temperature on Formation of the Plant Cysteine Synthase Complex

Temperatures above 35 °C reduce the demand for cysteine synthesis (Nieto-Sotelo and Ho 1986), but increased sulfur assimilation and cysteine production help maintain glutathione levels in response to chilling stress in plants (Kocsy et al. 2000, Gomez et al. 2004, Phartiyal et al. 2006). Interestingly, ITC analysis of the temperature-dependence of interaction between AtOASS and the C10 peptide demonstrates that above 35 °C changes occur in how the subunits respond to peptide binding (Kumaran and Jez 2007). At elevated temperatures, the binding of a C10

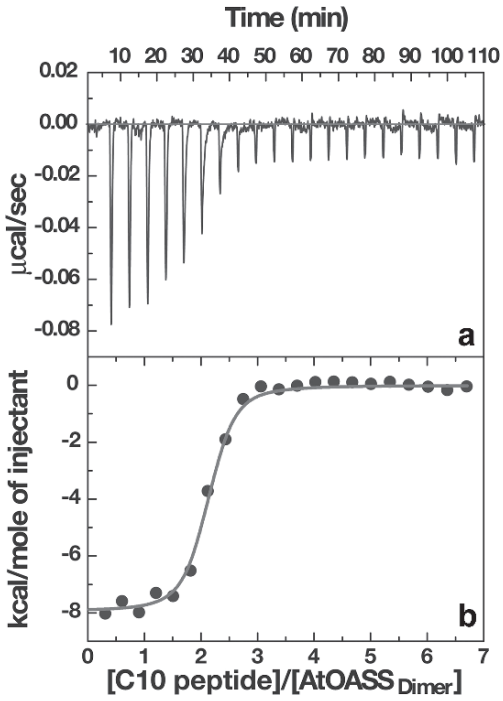

Fig. 5.4 (**a**) Titration of AtOASS with C10 peptide. ITC data is plotted as heat signal (µcal/sec) versus time (min). The experiment consisted of 20 injections of 12 µl each of C10 peptide (21.8 µM) into a solution containing AtOASS (0.65 µM) at 20 °C. (**b**) The integrated heat responses per injection from panel **a** are plotted as normalized heat per mole of injectant. The solid line represents the best fit to a two independent sites binding model.

peptide to one subunit decreases the affinity for the second subunit through an apparent negative cooperativity. The molecular origin of negative cooperativity is difficult to trace, but a reasonable explanation could be a conformation change that may have transformed the nonallosteric enzyme to an allosteric one. When the enzyme is in allosteric state, binding at one subunit may lead to closing of the active site in the other subunit. Thus, at higher temperatures, the activity of OASS in the plant cysteine synthase may be lost due to structural changes that occur as the enzyme transforms to a closed state from the open state upon complex formation. Transition between open and closed forms of the bacterial OASS occurs upon substrate binding (Rabeh and Cook 2004), but a similar transition has not been reported for the plant enzyme.

4.3 Thermodynamic Basis for Interaction of AtOASS and the C-Terminus of AtSAT

The temperature-dependence of thermodynamic parameters of a binding interaction also provides detailed information about the different forces that drive binding. The observed enthalpy change for binding of the C10 peptide by AtOASS increases

as temperature rises (Kumaran and Jez 2007). Moreover, the binding interaction is exothermic at all temperatures examined, which indicates a favorable negative enthalpy of binding. Overall, both enthalpy and entropy contribute to the binding free energy, although the relative contributions depend on the temperature. At lower temperatures (10 and 15 °C), the entropic contribution is more favorable, and this contribution decreases with increasing temperature. Interaction at these temperatures is driven by both enthalpy and entropy, but enthalpy becomes the dominant force at higher temperatures. The crystal structure of unbound AtOASS revealed that multiple water molecules occupy the active site (Bonner et al. 2005). Presumably, binding of the C10 peptide releases nonspecifically bound water molecules from AtOASS at lower temperatures, thereby increasing the net entropy of the system and contributing to the free energy of binding.

An important thermodynamic property of molecular interactions is derived from temperature-dependence of the observed enthalpy of interaction. The first derivative of temperature-dependence of enthalpy change, heat capacity (ΔC_p) for AtOASS-C10 complex, is ΔC_p = -0.401 ± 0.025 kcal mol^{-1} deg^{-1} (Kumaran and Jez 2007). The negative value of ΔC_p indicates that the interaction is specific and is accompanied by burial of nonpolar surface area. In addition, the negative ΔCp is responsible for the shift from a predominantly entropic contribution to an enthalpic one as the temperature rises. At 22 °C (T_S), the entropic contribution to the binding process is zero and binding process becomes completely driven by enthalpy at higher temperatures. On the other hand, at −3.0 °C (T_H), the net enthalpic contribution is ~0 kcal mol^{-1}, with the binding process driven completely by entropy. Thus, both enthalpy and entropy contribute favorably to the Gibbs energy of complex formation between −3.0 °C and 22 °C.

5 Conclusion

The series of structural and functional studies discussed here provide an initial view of the plant cysteine synthase complex and the molecular basis for interaction of SAT and OASS. Although the experimental approaches discussed here extensively examine protein-peptide interactions, the general strategy for dissecting how SAT and OASS interact will be applicable with the full-length proteins.

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References

Berkowitz O, Wirtz M, Wolf A, Kuhlmann J, Hell R (2002) Use of biomolecular interaction analysis to elucidate the regulatory mechanism of the cysteine synthase complex from *Arabidopsis thaliana*. J Biol Chem 277:30629-34

Bogdanova N, Hell R (1997) Cysteine synthesis in plants: protein-protein interactions of serine acetyltransferase from *Arabidopsis thaliana*. Plant J 11:251-62

Bonner ER, Cahoon RE, Knapke SM, Jez JM (2005) Molecular basis of cysteine biosynthesis in plants: structural and functional analysis of O-acetylserine sulfhydrylase from *Arabidopsis thaliana*. J Biol Chem 280:38803-13

Burkhard P, Rao GS, Hohenester E, Schnackerz KD, Cook PF, Jansonius JN (1998) Three-dimensional structure of O-acetylserine sulfhydrylase from *Salmonella typhimurium*. J Mol Biol 283:121-33

Campanini B, Speroni F, Salsi E, Cook PF, Roderick SL, Huang B, Bettati S, Mozzarelli A (2005) Interaction of serine acetyltransferase with O-acetylserine sulfhydrylase active site: evidence from fluorescence spectroscopy. Protein Sci 14:2115-24

Claus MT, Zocher GE, Maier, THP, Schulz GE (2005) Structure of the O-acetylserine sulfhydrylase isoenzyme CysM from *Escherichia coli*. Biochemistry 44:8620-6

Cook PF, Wedding RT (1977) Initial kinetic characterization of the multienzyme complex, cysteine synthetase. Arch Biochem Biophys 178:293-302

Droux M, Ruffet ML, Dounce R, Job B (1998) Interactions between serine acetyltransferase and O-acetylserine(thiol)lyase in higher plants: structural and kinetic properties of the free and bound enzymes. Eur J Biochem 255:235-45

Francois JA, Kumaran S, Jez JM (2006) Structural basis for interaction of O-acetylserine sulfhydrylase and serine acetyltransferase in the *Arabidopsis* cysteine synthase complex. Plant Cell 18:3647-55

Gomez LD, Vanacker H, Buchner P, Noctor G, Foyer CH (2004) Intercellular distribution of glutathione synthesis in maize leaves and its response to short-term chilling. Plant Physiol 134:1662-71

Hell R, Hillebrand H (2001) Plant concepts for mineral acquisition and allocation. Curr Opin Biotech 12:161-8

Hell R, Jost R, Berkowitz O, Wirtz M (2002) Molecular and biochemical analysis of the enzymes of cysteine biosynthesis in the plant *Arabidopsis thaliana*. Amino Acids 22:245-57

Hopkins L, Parmar S, Blaszczyk A, Hesse H, Hoefgen R, Hawkesford MJ (2005) O-acetylserine and the regulation of expression of genes encoding components for sulfate uptake and assimilation in potato. Plant Physiol 138:433-40

Huang B, Vetting MW, Roderick SL (2005) The active site of O-acetylserine sulfhydrylase is the anchor point for bienzyme complex formation with serine acetyltransferase. J Bacteriol 187:3201-5

Johnson CM, Huang B, Roderick RL, Cook PF (2004a) Kinetic mechanism of the serine acetyltransferase from *Haemophilus influenzae*. Arch Biochem Biophys 429:115-22

Johnson CM, Huang B, Roderick RL, Cook PF (2004b) Chemical mechanism of the serine acetyltransferase from *Haemophilus influenzae*. Biochemistry 49:15534-9.

Kocsy G, Szalai G, Vagujfalvi A, Stehli L, Orosz G, Galiba G (2000) Genetic study of glutathione accumulation during cold hardening in wheat. Planta 210:295-301

Kopriva S (2006) Regulation of sulfate assimilation in *Arabidopsis* and beyond. Ann Bot 97:479-95

Koprivova A, Suter M, den Camp RO, Brunold C, Kopriva S (2000) Regulation of sulfate assimilation by nitrogen in *Arabidopsis*. Plant Physiol 122:737-46

Kredich NM, Becker MA, Tomkins GM (1969) Purification and characterization of cysteine synthetase, a bifunctional protein complex, from *Salmonella typhimurium*. J Biol Chem 244:2428-39

Kumaran S, Jez JM (2007) Thermodynamics of the interaction between O-acetylserine sulfhydrylase and the C-terminus of serine acetyltransferase. Biochemistry 46:5586-94

Leu LS, Cook PF (1994) Kinetic mechanism of serine transacetylase from *Salmonella typhimurium*. Biochemistry 33:2667-71

Liszewska F, Lewandowska M, Plochocka D, Sirko A (2007) Mutational analysis of O-acetylserine (thiol) lyase conducted in yeast two-hybrid system. Biochim Biophys Acta 1774:450-5

Malmquist M (1993) Biospecific interaction analysis using biosensor technology. Nature 361:186-7

Mino K, Yamanoue T, Sakiyama T, Eisaki N, Matsuyama A, Nakanishi K (1999) Purification and characterization of serine acetyltransferase from *Escherichia coli* partially truncated at the C-terminal region. Biosci Biotech Biochem 63:168-79

Mino K, Hiraoka K, Imamura K, Sakiyama T, Eisaki N, Matsuyama A, Nakanishi K (2000) Characteristics of serine transacetylase from *Escherichia coli* deleting different lengths of amino acid residues from the C-terminus. Biosci Biotech Biochem 64:1874-80

Nakamura K, Tamura G (1990) Isolation of serine acetyltransferase complexed with cysteine synthase from *Allium tuberosum*. Agric Biol Chem 54:649-56

Nieto-Sotelo J, Ho TH (1986) Effect of heat shock on the metabolism of glutathione in maize roots. Plant Physiol 82:1031-5

Noji M, Inoue K, Kimura N, Gouda A, Saito K (1998) Isoform-dependent differences in feedback regulation and subcellular localization of serine acetyltransferase involved in cysteine biosynthesis from *Arabidopsis thaliana*. J Biol Chem 273:32739-45

Olsen LR, Huang B, Vetting MB, Roderick SL (2004) Structure of serine acetyltransferase in complexes with CoA and its cysteine feedback inhibitor. Biochemistry 43:6013-19

Phartiyal P, Kim WS, Cahoon RE, Jez JM, Krishnan HB (2006) Soybean ATP sulfurylase, a homodimeric enzyme involved in sulfur assimilation, is abundantly expressed in roots and induced by cold treatment. Arch Biochem Biophys 450:20-9

Pye VE, Tingey AP, Robson RL, Moody PCE (2004) The structure and mechanism of serine acetyltransferase from *Escherichia coli*. J Biol Chem 279:40729-36

Rabeh WM, Cook PF (2004) Structure and mechanism of O-acetylserine sulfhydrylase. J Biol Chem 279:26803-6

Saito K (2004) Sulfur assimilatory metabolism: the long and smelling road. Plant Physiol 136:2443-50

Smith FW, Hawkesford MJ, Ealing PM, Clarkson DT, Vandenberg PS, Belcher AR, Warrilow AG (1997) Regulation of expression of a cDNA from barley roots encoding a high-affinity sulfate transporter. Plant J 12:875-8

Stites WE (1997) Protein-protein interactions: interface structure, binding thermodynamics, and mutational analysis. Chem Rev 97:1233-50

Tai CH, Burkhard P, Gani D, Jenn T, Johnson C, Cook PF (2001) Characterization of the allosteric anion-binding site of O-acetylserine sulfhydrylase. Biochemistry 40:7446-52

Wirtz M, Berkowitz O, Droux M, Hell R (2001) The cysteine synthase complex in plants: mitrochondrial serine acetyltransferase from *Arabidopsis thaliana* carries a bifunctional domain for catalysis and protein-protein interaction. Eur J Biochem 268:686-93

Wirtz M, Droux M (2005) Synthesis of the sulfur amino acids: cysteine and methionine. Photosynth Res 86:345-6

Wirtz M, Hell R (2007) Dominant-negative modification reveals the regulatory function of the multimeric cysteine synthase protein complex in transgenic tobacco. Plant Cell 19:625-39

Zhao C, Moriga Y, Feng B, Kumada Y, Imanaka H, Imamura K, Nakanishi K (2006) On the interaction site of serine acetyltransferase in the cysteine synthase complex from *Escherichia coli*. Biochem Biophys Res Comm 341:911-16